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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.: 09/870,414  
Applicant(s): Anton-Lewis Usala  
Filed: May 30, 2001  
Art Unit: 1654  
Examiner: Gupta, Anish  
Title: METHOD OF TREATING CHRONIC ULCERS  
Confirmation No.: 7087  
Docket No.: 035626/234825  
Customer No.: 00826

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**APPEAL BRIEF UNDER 37 CFR § 41.37**

This Appeal Brief is filed pursuant to the "Notice of Appeal to the Board of Patent Appeals and Interferences" filed November 14, 2005.

1. ***Real Party in Interest.***

The real party in interest in this appeal is Encelle, Inc., the assignee of the above-referenced patent application.

2. ***Related Appeals and Interferences.***

There are no related appeals and/or interferences involving this application or its subject matter.

3. ***Status of Claims.***

Claims 1-55 are pending and all claims stand rejected as unpatentable over two separate combinations of three prior art references as set forth in greater detail below. Additionally, claims 1-29, 31-42, 46-48, 50, 51, 54, and 55 stand rejected under an obviousness-type double patenting rejection over a combination of three references. All three rejections are appealed herein.

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4. ***Status of Amendments.***

All claim amendments presented during prosecution were entered and are set forth in the clean copy of the pending claims appended to the brief. Claims 1, 32, and 48 have been amended once during prosecution. Claims 52-55 were added during prosecution.

5. ***Summary of Claimed Subject Matter.***

It has been discovered that the hydrogel matrix set forth in the claims is capable of successfully treating and healing chronic ulcers, such as ulcers resulting from diabetes-related vasculoneuropathy. Although ulcers of this type are often resistant to conventional wound treatments, the method of the present invention can heal chronic lesions or ulcers in a matter of days or weeks. The present invention typically involves the administration of a therapeutic amount of a hydrogel matrix comprising gelatin and a long chain carbohydrate to the ulcer in a manner that exposes polar groups of the basement membrane of the patient's tissue to the components of the matrix (e.g., by injection).

Independent claim 1 is directed to a method of treating an ulcer, the method comprising administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising gelatin and a long chain carbohydrate. The gelatin component of the matrix is described on pages 6-7 of the application and the long chain carbohydrate component is described on page 7. Preferred matrix ingredient concentrations and a method of making the matrix are set forth on page 9, and an exemplary treatment/administration protocol is set forth on pages 10 and 11. An illustrative list of ulcers that can be treated is set forth on page 6.

Independent claim 32 also recites a method of treating an ulcer, the method comprising administering a therapeutic amount of a hydrogel matrix to the ulcer, the matrix composition comprising denatured collagen, dextran, and an effective amount of polar amino acids selected from the group consisting of arginine, lysine, histidine, glutamic acid, aspartic acid, and mixtures thereof. Denatured collagen is a form of gelatin, and is discussed on page 6. Dextran is an exemplary carbohydrate discussed on page 7. The addition of polar amino acids is described on pages 7-8, and their effect is illustrated in Fig. 3.

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Independent claim 48 is directed to a method of treating an ulcer comprising administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising denatured collagen, dextran, L-cysteine, and an effective amount of polar amino acids selected from the group consisting of arginine, lysine, histidine, glutamic acid, aspartic acid, and mixtures thereof. The use of cysteine as an additive is set forth on page 8.

Finally, independent claim 52 recites a method of treating an ulcer, wherein the method comprises administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising gelatin, a long chain carbohydrate having a molecular weight of about 20,000 to about 1,000,000 Daltons, and at least one polar amino acid, wherein said administering step comprises injecting the matrix into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer, and combinations thereof. Suggested injection locations are described on pages 10 and 11, and in Example 3.

**6. *Grounds of Rejection to be Reviewed on Appeal.***

All claims remain rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of either U.S. Patent No. 6,231,881 to Usala or PCT Application No. WO 00/02999 to Usala in view of the Miller reference (J. Dermatol. Surg. Oncol., 1993), and in further view of either the Davis (U.S. 5,487,899) or Pickart (U.S. 5,059,588) patents. Additionally, a majority of the claims remain rejected under the judicially created doctrine of obviousness-type double patenting as being patentable over the claims of U.S. Patent No. 6,261,587 in view of the Miller reference, and in further view of either Davis or Pickart. The '881 patent, the '587 patent, and the cited PCT application are collectively referred to as "the Usala references" hereinbelow. Appellant respectfully requests that the Board overturn all three rejections for the reasons set forth below.

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**7. Argument.**

The Examiner argues that the Miller reference implies that "granulation and reepithelialization of an ulcer is desired," and further relies upon the Miller reference as teaching that agents that encourage wound healing can be used in the treatment of ulcers. The Examiner concludes that vascularization would be viewed, based on the Miller reference, as a beneficial treatment of ulcers because it is a "beneficial effect in granulation of tissue formation." The Examiner relies upon the Davis and Pickart references as teaching that blood vessel formation is an aspect of granulation tissue formation.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation either in the references themselves, or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim elements. The suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on the applicant's disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

It is respectfully submitted that the Examiner has failed to establish a *prima facie* case of obviousness necessary to support all three of the rejections of record. In particular, the Examiner has failed to show a proper suggestion or motivation to combine the references in the manner contemplated by the rejection, failed to show a reasonable expectation of success, and failed to provide a combination of references that teach every claim limitation of every rejected claim.

**I. Lack of Motivation to Combine**

There is nothing in the cited art to suggest the combination of any of the Usala references with the Miller reference. As admitted by the Examiner, the '881 patent is entirely silent as the treatment of any sort of ulcer. Rather, the Usala '881 patent is directed to a device for release of hormones. The '881 patent merely suggests that the matrix composition can increase vascularization. Similarly, the cited Usala PCT application is directed to a matrix for long-term proliferation of cells and simply mentions that the matrix may promote vascularization at a transplant site. There is no mention of treatment of any type of ulcer. The Usala '587 patent

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claims a method of stimulating vascularization, but does not mention treatment of ulcerated tissue. Thus, there is clearly no teaching in the Usala references that might motivate one of ordinary skill in the art to combine those references with the Miller reference.

The Miller reference also fails to provide the necessary motivation to combine, and in particular, cannot be fairly viewed as motivating the use of the matrix of the Usala references in an ulcer treatment protocol. The Miller reference stresses the need for separate treatment protocols for the two main classes of diabetes-related ulcers; namely, ischemic ulcers and neuropathic ulcers. The Miller reference describes the ischemic ulcer as "secondary to vascular compromise" and notes that "without reconstructive vascular surgery," the chances of healing is poor and amputation inevitable (page 759, second column, first full paragraph) (emphasis added). Similarly, the Miller reference suggests that an ischemic foot can be treated with vein graft reconstruction (page 760, first paragraph).

With respect to neuropathic ulcers, the Miller reference actually teaches away from vascularization-related treatments by suggesting that it is mistaken to consider occlusive small vessel disease as having a significant role in chronic nonhealing neuropathic ulcers (page 759, last paragraph). Miller suggests that "intrinsic microvascular disease" is not a major etiologic factor in the neuropathic foot (page 760, first paragraph).

Thus, the Miller reference merely suggests that in certain ulcers, specifically ischemic ulcers, vascular repair in the form of reconstructive vascular surgery is required to avoid amputation. For the treatment of neuropathic ulcers, the Miller reference suggests tissue debridement and relief from pressure at the site of the ulcer and teaches away from vascular-related therapies.

As noted above, at best, the Usala references can be viewed as suggesting that the matrix described therein can promote formation of microvasculature. However, the Miller reference fails to teach or suggest that a treatment involving microvascular formation would be suitable for ulcer treatment. With respect to neuropathic ulcers, the Miller reference expressly discounts the suggestion that microvascular disease is an etiologic factor and, thus, teaches away from vascular-related treatments. The "vascular repair" language relied upon by the Examiner is only used by Miller in reference to the treatment of ischemic ulcers secondary to major vascular compromise in the foot. Miller stresses that the type of vascular repair that is required is

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reconstructive vascular surgery, and there is nothing in the Miller reference that would suggest that a microvasculature-forming matrix would be helpful in the least. Thus, there is no teaching or suggestion in the Miller reference, when considered singly or in combination with the Usala references, that a matrix comprising gelatin and a long chain carbohydrate would be suitable for ulcer treatment.

In response to Appellant's previous arguments, the Examiner has taken the position that the Miller article teaches that an ulcer heals through tissue granulation and reepithelialization and has alleged that the Miller reference suggests numerous agents that can be administered to the ulcer to provide an optimal environment for healing. The Examiner goes on to allege that the Miller reference teaches that antibiotics and growth factors result in the stimulation of granulation of tissue, and that it is well known that granulation of tissue consists of new blood vessel formation, fibroblast activity, and reepithelialization, as evidenced by Davis or Pickart. The Examiner concludes that one of ordinary skill in the art would be motivated by the Miller reference to use the hydrogel matrix of the Usala references of record to treat diabetic foot ulcers because, based on the prior art, "one would expect vascularization to lead to granulation of the tissue and ultimate treatment of the ulcer." Appellant respectfully traverses this line of reasoning, and particularly traverses the statement quoted above.

Appellant respectfully submits that the Miller reference would not be viewed by one of ordinary skill in the art as providing motivation to use the hydrogel matrix described in the Usala references in the treatment of a diabetic ulcer. While the Examiner is correct that page 761 of the Miller reference contains a single sentence that addresses granulation tissue and reepithelialization, the reference only mentions those effects as a consequence of surgical debridement of necrotic tissue. This specific sentence referred to on page 761 by the Examiner states that "[n]ecrotic tissue and callus must be completely excised to provide a clean ulcer base in preparation for a granulation tissue and reepithelialization." The only motivation that this sentence provides to one skilled in the art is to utilize tissue debridement as a means for preparing an ulcer for granulation tissue and reepithelialization. There is no mention on page 761 of any other ulcer treatment that is designed to have any effect on granulation tissue or reepithelialization. Thus, the teachings on page 761 are irrelevant to the presently claimed invention.

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The teachings relied upon by the Examiner on page 762 are likewise irrelevant to the present invention. The Examiner states that the Miller reference teaches that "antibiotics and growth factors" result in stimulation of granulation of tissue. However, this is not entirely accurate. Page 762 of the Miller reference teaches that a moist environment promotes reepithelialization. For that reason, the Miller reference teaches the use of moist saline dressings, the use of antibiotics to prevent desiccation of the wound base, and benzoyl peroxide to promote a moist antiseptic wound bed. The Miller reference focuses on treatments designed to keep the wound moist and never states that antibiotics directly lead to reepithelialization or granulation tissue. Page 762 of Miller does state that benzoyl peroxide "might" stimulate granulation tissue, but such an ambivalent statement can hardly be expected to motivate one of ordinary skill in the art in any way meaningful to the present invention. The only teaching in Miller regarding growth factors is a single sentence that states that such factors attract fibroblasts and other cells involved in the early phases of wound healing. The reference does not state that growth factors lead to tissue granulation.

Thus, it is respectfully submitted that the Examiner appears to have described the teachings of the Miller reference in overly broad terms. There is clearly no express teaching in the Miller reference that would suggest that an agent that increases vascularization would lead to granulation of tissue and ultimate treatment of an ulcer. In fact, the Miller reference fails to suggest or describe any ulcer treatments that lead to vascularization and does nothing to indicate that such treatments would be helpful.

The Examiner goes on to rely upon either the Davis or Pickart patents as teaching that granulation of tissue consists of new blood vessel formation, fibroblast activity, and reepithelialization. However, these teachings fail to address the deficiencies of the Miller reference. Davis and Pickart merely describe the cascade of events involved in wound healing and note that formation of blood vessels is only one aspect of granulation tissue formation. The Davis and Pickart references suffer from the same deficiency as Miller. There is nothing in either Davis or Pickart to suggest that stimulation of microvasculature by itself will stimulate wound healing. As noted in both references, granulation tissue formation involves other processes, such as fibroblast activity and reepithelialization. There is no suggestion in either reference that stimulation of blood vessel formation alone will automatically trigger the

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remaining wound healing processes (i.e., fibroblast activity or reepithelialization) that are necessary for tissue granulation. Thus, the art of record simply does not support the Examiner's statement that vascularization would be expected to lead to tissue granulation.

In sum, Appellant again emphasizes that none of the references of record teach or suggest that improving microvasculature would necessarily result in stimulation of the wound healing process, particularly in the context of chronic ulcer treatment. The Miller reference does not even mention microvasculature formation at all. In light of these deficiencies of the cited art, Appellant respectfully requests that the Board overturn all rejections noted above.

Appellants also note that the Examiner twice mentions that Appellant's arguments address distinctions not found in the claims. First, in the office action dated 11/30/04, the Examiner notes that Appellants make a distinction between neuropathic ulcers and ischemic ulcers, but opines that the distinction is not embodied in the claims. Second, in the advisory action dated 12/30/05, the Examiner states that the claims are open to any ulcer, but Appellant's arguments are concentrated on diabetic foot ulcers. It is not clear to Appellant why the Examiner has raised these points. The claims of record include claims that more specifically recite treatment of ulcers resulting from diabetes-related vasculoneuropathy (claim 53) and treatment of diabetic foot ulcers (claim 46). However, the Examiner has applied the same rejections to these claims. If the Examiner views such claims as patentable over the art, it is requested that the Examiner indicate their allowability on the record.

## **II. Lack of Reasonable Expectation of Success**

Appellant also respectfully submits that the Examiner has failed to establish that one of ordinary skill in the art would have a reasonable expectation of success if the references of record are combined as contemplated in the rejections. Appellant has submitted credible evidence that strongly suggests that the Examiner has not, and in fact cannot, show the requisite reasonable expectation of success needed to support the Examiner's contention that the combined teachings of the references motivate one to use any vascularizing agent to treat diabetic foot ulcers.



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The Examiner cannot point to so much as a single sentence in the Miller reference that directly suggests that an agent that encourages vascularization would be beneficial in an ulcer treatment regime. Instead, the Examiner has seized upon statements in Miller regarding the desirability of tissue granulation and boldly proclaimed, in essence, that the art suggest that any agent that encourages vascularization would be beneficial in ulcer treatment. In an attempt to further bolster this claim, the Examiner points to Davis and Pickart, which merely teach that blood vessel formation is one aspect of granulation tissue formation.

The art relied upon by the Examiner simply fails to credibly suggest that a vascularization-inducing agent would have a reasonable expectation of success as an ulcer treatment. There is no reference of record directly connecting a vascularization effect with successful treatment of an ulcer. There is also no reference of record that suggests that encouraging vascularization will lead to granulation of tissue. The Pickart and Davis references only teach that blood vessel formation is one event that characterizes granulation of tissue. There is nothing to suggest in either reference that encouraging blood vessel formation will result in tissue granulation, meaning that the vascularization effect will necessarily also trigger the remaining wound healing processes, such as fibroblast activity or reepithelialization, that are necessary for tissue granulation. The art of record simply does not support the Examiner's statement that inducement of vascularization, by itself, would lead to tissue granulation or would be reasonably expected to be successful as an ulcer treatment.

If the Examiner's contention is correct, which Appellant does not admit, then one of ordinary skill in the art would view any vascularizing agent as a beneficial treatment for ulcers. However, the state of the art is clearly inconsistent with this conclusion. In fact, there is at least one known vascularizing agent that has been tried as a diabetic foot ulcer treatment and failed. In Chapter 18 of Levin and O'Neal's "The Diabetic Foot" (6<sup>th</sup> Edition), David Steed notes in Chapter 18 (entitled *Modulating Wound Healing in Diabetes*) that fibroblast growth factors (FGFs) "act as angiogenesis factors by stimulating growth of new blood vessels through proliferation of capillary endothelial cells" (p. 399). However, Steed goes on to admit that there are "no clinical trials that have proven FGF to be of benefit in clinical wound healing."

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In addition, Appellants direct attention to the Richard *et al.* reference appearing in a 1995 issue of the journal *Diabetes Care*. In the Richard reference, a study of the use of FGF as a treatment for chronic neuropathic foot ulcers in diabetic patients is described. As noted on the first page (p. 64), the researchers noted that FGF performed no better than a placebo in reducing ulcer perimeter and area, and concluded that application of FGF has no advantage over a placebo for healing chronic neuropathic diabetic ulcers of the foot. Thus, Appellants have presented prior art evidence that is clearly contrary to the Examiner's conclusion that any vascularizing agent would be expected to succeed as an ulcer treatment. In fact, FGF is a vascularizing growth factor that has failed as a diabetic foot ulcer treatment. This clearly establishes that one of ordinary skill in the art, having benefit of the knowledge of the FGF failure, would not expect each and every agent capable of triggering vascularization to automatically find success as an ulcer treatment. In fact, one of ordinary skill in the art would be highly skeptical of such a claim. As a result, *prima facie* obviousness clearly cannot be established using the rationale and combination of references relied upon by the Examiner.

In the advisory action, the Examiner pointed to two other studies mentioned on page 67 in the Richard reference that supposedly support his claim. In one, a complex mixture of numerous ingredients significantly improved healing of chronic diabetic ulcers. However, no conclusion related to the rejections of record can be drawn from this study since there is no way to determine if the perceived healing effect is at all linked to a vascularizing agent. In the second study noted by the Examiner, bFGF was reported to enhance wound healing "slightly, but nonsignificantly" (emphasis added). The Examiner conveniently left out the word "nonsignificantly" from his description in the advisory action. This study hardly supports the rejections of record, particularly in light of the abysmal findings of the main study reported in the Richard reference. Viewing the Richard reference as a whole, the inescapable conclusion is that the use of growth factors such as bFGF to treat chronic diabetic wounds is nothing more than unproven and "controversial" (page 67, middle column), and even the authors conclude that the profound healing defect of diabetes may explain the failure of bFGF to demonstrate beneficial effect (page 68).

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When viewed in its entirety, the available art simply does not support the Examiner's obviousness rationale. There is nothing that would render obvious the use of the vascularizing agent of the Usala references in the treatment of a diabetic ulcer, and in particular, there is nothing to provide one of ordinary skill in the art with the reasonable expectation of success required to support the rejections of record.

### **III. Failure to Teach or Suggest All Claim Limitations**

Claims 45, 49, 52, and 53 recite that the matrix is injected into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer and combinations thereof. Claim 54 recites that one or more injections of the matrix are made in the area of the dermal/subdermal tissue junction. None of the references of record teach or suggest injecting an ulcer with a hydrogel matrix, and certainly fail to suggest any of the specific injection locations set forth in the above claims. Further, the Examiner has not adequately explained how the references of record can be applied to the above claims. For this additional reason, Appellant respectfully requests overturning the rejection of the above-referenced claims.

#### **8. *Claims Appendix.***

An appendix containing a copy of the claims involved in the appeal is attached.

#### **9. *Evidence Appendix.***

Two literature references have been submitted to the Examiner, and are discussed herein: Richard *et al.*, *Diabetes Care*, Vol. 18, No. 1, pages 64-69 (1995) and The Diabetic Foot, Sixth Ed., Chapter 18, pages 395-403 (2001). Copies are enclosed.

#### **10. *Related Proceedings Appendix.***

There are no decisions by a court or the Board in related proceedings.

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**CONCLUSION**

In view of the foregoing arguments, Appellant respectfully submits that Claims 1-55 are patentable over the cited references. A decision from the Board of Patent Appeals and Interferences reversing the final rejection of the pending claims is therefore earnestly solicited.

Respectfully submitted,

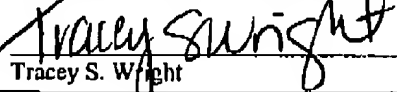


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**CLAIMS APPENDIX**

1. (Previously Presented) A method of treating an ulcer, comprising administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising gelatin and a long chain carbohydrate.
2. (Original) The method of Claim 1, wherein the matrix comprises about 0.01 to about 40 mM gelatin.
3. (Original) The method of Claim 1, wherein the gelatin comprises denatured collagen.
4. (Original) The method of Claim 1, wherein the long chain carbohydrate comprises dextran.
5. (Original) The method of Claim 4, wherein the matrix comprises about 0.01 to about 10 mM dextran.
6. (Original) The method of Claim 1, wherein the long chain carbohydrate has a molecular weight of about 20,000 to about 1,000,000 Daltons.
7. (Original) The method of Claim 1, wherein the matrix further comprises an effective amount of polar amino acids selected from the group consisting of arginine, lysine, histidine, glutamic acid, and aspartic acid.
8. (Original) The method of Claim 7, wherein the effective amount of polar amino acids comprises about 3 to about 150 mM of polar amino acids.
9. (Original) The method of Claim 7, wherein the effective amount of polar amino acids comprises about 10 to about 65 mM of polar amino acids.

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10. (Original) The method of Claim 7, wherein the polar amino acids are selected from the group consisting of arginine, glutamic acid, lysine and mixtures thereof.

11. (Original) The method according to claim 10, wherein the matrix comprises:

about 2 to about 60 mM of L-glutamic acid;  
about 0.5 to about 30 mM of L-lysine; and  
about 1 to about 40 mM of arginine.

12. (Original) The method of Claim 11, wherein the matrix comprises:

about 5 to about 40 mM of L-glutamic acid;  
about 1 to about 15 mM of L-lysine; and  
about 1 to about 30 mM of arginine.

13. (Original) The method according to claim 10, wherein the effective amount of polar amino acids comprises about 2 to about 60 mM of L-glutamic acid.

14. (Original) The method according to claim 10, wherein the effective amount of polar amino acids comprises about 1 to about 40 mM of arginine.

15. (Original) The method of Claim 10, wherein the effective amount of polar amino acids comprises about 0.5 to about 30 mM of L-lysine.

16. (Original) The method of Claim 1, wherein the matrix further comprises at least one nitric oxide inhibitor.

17. (Original) The method of Claim 16, wherein the nitric oxide inhibitor is selected from the group consisting of L-cysteine, L-arginine analogues, cystine, heparin, and mixtures thereof.

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18. (Original) The method of Claim 16, wherein the nitric oxide inhibitor is present in an amount of about 5 to about 1000  $\mu\text{M}$ .

19. (Original) The method of Claim 16, wherein the nitric oxide inhibitor is present in an amount of about 20 to about 200  $\mu\text{M}$ .

20. (Original) The method of Claim 1, wherein the matrix further comprises about 5 to about 500  $\mu\text{M}$  of L-cysteine.

21. (Original) The method of Claim 20, wherein the matrix comprises about 15 to about 25  $\mu\text{M}$  of L-cysteine.

22. (Original) The method of Claim 1, wherein the matrix further comprises about 5 to about 500  $\mu\text{M}$  of an L-arginine analogue.

23. (Original) The method of Claim 22, wherein the L-arginine analogue comprises aminoguanidine.

24. (Original) The method of Claim 22, wherein the matrix comprises about 15 to about 25  $\mu\text{M}$  of an L-arginine analogue.

25. (Original) The method of Claim 1, wherein the matrix further comprises a superoxide inhibitor.

26. (Original) The method of Claim 25, wherein the superoxide inhibitor comprises EDTA or a salt thereof.

27. (Original) The method of Claim 25, wherein the superoxide inhibitor is present in an amount of about 1 to about 8 mM.

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28. (Original) The method of claim 1, wherein the gelatin comprises denatured collagen and the long chain carbohydrate comprises dextran.

29. (Original) The method of Claim 1, wherein said administering step comprises injecting the matrix into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer and combinations thereof.

30. (Original) The method of Claim 1, wherein the therapeutic amount comprises about 1.0 to about 60 ml.

31. (Original) The method of Claim 1, wherein the ulcer is a diabetic foot ulcer.

32. (Previously presented) A method of treating an ulcer, comprising administering a therapeutic amount of a hydrogel matrix to the ulcer, the matrix composition comprising denatured collagen, dextran, and an effective amount of polar amino acids selected from the group consisting of arginine, lysine, histidine, glutamic acid, aspartic acid, and mixtures thereof.

33. (Original) The method of Claim 32, wherein the effective amount of polar amino acids comprises about 3 to about 150 mM of polar amino acids.

34. (Original) The method of Claim 33, wherein the effective amount of polar amino acids comprises about 10 to about 65 mM of polar amino acids.

35. (Original) The method of Claim 32, wherein the polar amino acids are selected from the group consisting of arginine, glutamic acid, lysine and mixtures thereof.

36. (Original) The method according to claim 35, wherein the matrix comprises:  
about 2 to about 60 mM of L-glutamic acid;  
about 0.5 to about 30 mM of L-lysine; and



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about 1 to about 40 mM of arginine.

37. (Original) The method of Claim 32, wherein the matrix further comprises at least one nitric oxide inhibitor.

38. (Original) The method of Claim 37, wherein the nitric oxide inhibitor is selected from the group consisting of L-cysteine, L-arginine analogues, cystine, heparin, and mixtures thereof.

39. (Original) The method of Claim 37, wherein the nitric oxide inhibitor is present in an amount of about 5 to about 1000  $\mu$ M.

40. (Original) The method of Claim 37, wherein the nitric oxide inhibitor is present in an amount of about 20 to about 200  $\mu$ M.

41. (Original) The method of Claim 32, wherein the matrix further comprises about 5 to about 500  $\mu$ M of L-cysteine.

42. (Original) The method of Claim 32, wherein the matrix further comprises about 5 to about 500  $\mu$ M of an L-arginine analogue.

43. (Original) The method of Claim 32, wherein the matrix further comprises a superoxide inhibitor.

44. (Original) The method of Claim 43, wherein the superoxide inhibitor comprises EDTA or a salt thereof.

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45. (Original) The method of Claim 32, wherein said administering step comprises injecting the matrix into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer and combinations thereof.

46. (Original) The method of Claim 32, wherein the ulcer is a diabetic foot ulcer.

47. (Original) The method of Claim 32, wherein said therapeutic amount comprises about 1.0 ml to about 60 ml.

48. (Previously presented) A method of treating an ulcer, comprising administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising denatured collagen, dextran, L-cysteine, and an effective amount of polar amino acids selected from the group consisting of arginine, lysine, histidine, glutamic acid, aspartic acid, and mixtures thereof.

49. (Original) The method of Claim 48, wherein said administering step comprises injecting the matrix into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer and combinations thereof.

50. (Original) The method of Claim 48, wherein said therapeutic amount comprises about 1.0 mL to about 60 mL.

51. (Original) The method of Claim 48, wherein the ulcer is a diabetic foot ulcer.

52. (Previously presented) A method of treating an ulcer, comprising administering a therapeutic amount of a hydrogel matrix in liquid form to the ulcer, the matrix composition comprising gelatin, a long chain carbohydrate having a molecular weight of about 20,000 to about 1,000,000 Daltons, and at least one polar amino acid, wherein said administering step

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comprises injecting the matrix into one or more locations selected from the group consisting of locations within the ulcer, locations around the periphery of the ulcer, locations underneath the ulcer, and combinations thereof.

53. (Previously presented) The method of Claim 52, wherein the ulcer is a foot ulcer resulting from diabetes-related vasculoneuropathy.

54. (Previously presented) The method of Claim 1, wherein said administering step comprises one or more injections of the matrix in the area of the dermal/subdermal tissue junction.

55. (Previously presented) The method of Claim 1, further comprising the step of debriding the ulcer prior to said administering step.

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**EVIDENCE APPENDIX**

Two literature references have been submitted and are discussed herein: Richard *et al.*, *Diabetes Care*, Vol. 18, No. 1, pages 64-69 (1995) and The Diabetic Foot, Sixth Ed., Chapter 18, pages 395-403 (2001).

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**RELATED PROCEEDINGS APPENDIX**

There are no decisions by a court or the Board in related proceedings.

## ORIGINAL ARTICLE

# Effect of Topical Basic Fibroblast Growth Factor on the Healing of Chronic Diabetic Neuropathic Ulcer of the Foot

A pilot, randomized, double-blind, placebo-controlled study

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**OBJECTIVE** — To assess the efficacy and safety of topical human recombinant basic fibroblast growth factor (bFGF) on the healing of diabetic neuropathic foot ulcers.

**RESEARCH DESIGN AND METHODS** — Seventeen diabetic patients suffering from chronic neuropathic ulcer of the plantar surface of the foot entered a pilot, randomized, double-blind study comparing local application of bFGF with placebo. Main inclusion criteria were a typical neuropathic ulcer of Wagner grade I-III, more than 0.5 cm in the largest diameter, with an abnormally high vibration perception threshold in the absence of significant peripheral vascular disease or wound infection. bFGF or placebo was applied daily during the 6 weeks as inpatients then twice a week for 12 weeks. Evolution of ulcer size was assessed through weekly clinical examination and computerized photographs.

**RESULTS** — In the bFGF group, three of nine ulcers healed compared with five of eight in the placebo group (NS). The weekly reduction in ulcer perimeter and area was identical in both groups, as was the rate of linear advance from entry to the 6th week of treatment (bFGF:  $0.053 \pm 0.048$  mm vs. placebo:  $0.116 \pm 1.129$  mm); the same result was obtained at the 11th week. Moreover, percent healed area at the end of the study did not differ significantly. No side effects were observed during bFGF application.

**CONCLUSIONS** — Topical application of bFGF has no advantage over placebo for healing chronic neuropathic diabetic ulcer of the foot. Because diabetes causes significant wound-healing defects, we hypothesized that using a single growth factor might be insufficient to accelerate wound closure of diabetic ulcers.

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bFGF, basic fibroblast growth factor; ABI, ankle-brachial pressure index; PDWHP, platelet-derived wound healing formula; PDGF, platelet-derived growth factor.

Foot lesions are a major problem in people with diabetes mellitus, in terms of morbidity, mortality, disability, and financial cost (1). In diabetic patients, nonhealing foot ulcers, mainly due to peripheral and autonomic neuropathy (2-5), frequently lead to lower limb amputations (6,7). Numerous approaches using topical treatment have been proposed, including antibacterial agents (antibiotics, antiseptics), various types of dressings, powders, pastes, and creams (2,3). But most ulcers respond poorly to this conventional treatment, often requiring a long hospital stay (8), and the recurrence rate is high (9).

Wound healing is a complex process involving highly regulated responses of specialized cell types, in which locally secreted growth factors seem to play a key role (10). Recently, the availability of large quantities of growth factors has allowed researchers to test their therapeutic use as topical agents to accelerate wound healing (11).

Considering the impairment of wound healing in diabetes mellitus and the role of basic fibroblast growth factor (bFGF) in the healing process clearly suggested by experimental *in vivo* study (12,13), we conducted for the first time a pilot (phase I and II) randomized, double-blind, and placebo-controlled study to evaluate the efficacy and safety of topically applied recombinant bFGF in stimulating the repair of diabetic neuropathic foot ulcers.

## RESEARCH DESIGN AND METHODS

After obtaining informed consent, 17 diabetic patients aged 39 to 73 years, were entered into the study. They were recruited from two departments of diabetology at Montpellier University Hospital ( $n = 7$ ) and at Le Grau du Roi Medical Center ( $n = 10$ ). All but one had non-insulin-dependent diabetes mellitus. The experimental protocol in accordance with the Declaration of Helsinki was approved by the French National Ethical Committee.

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Patients were included if they had a typical, chronic, nonhealing, neuropathic ulcer on the plantar surface of the foot, grade 1-III, according to Wagner's classification (14); this classification system was chosen because it appears valuable in evaluating the outcome of diabetic foot ulcers (15). After mechanical excision, the largest part of the wound had to measure more than 0.5 cm. The neuropathic nature of the ulcers was confirmed by biothesiometry (16) with a vibration perception threshold higher than 30 V either at the great toe or at the medial malleolus (Biomedical, Newbury, OH). Moreover, no patient had significant peripheral vascular disease, as evidenced by Doppler wave form analysis; ankle-brachial pressure index (ABI) was not used, as most neuropathic patients had falsely elevated ABI values due to medial arterial calcification (17). Before patients entered the study, active infection was ruled out by clinical examination and X rays, and a tight glycemic control was obtained by intensive insulin therapy using three subcutaneous injections a day or continuous subcutaneous insulin infusion (Mark II Infusers, Novo Nordisk, Boulogne-Billancourt, France); intensive insulin therapy was maintained during the entire experimental period and patients were instructed to check their capillary blood glucose level three to six times a day.

After randomization, patients were treated with either placebo (normal saline) or bFGF (Famitralia Carlo Erba, Milano, Italy) until the ulcer was completely healed or the patient completed the 18 weeks of the treatment. The treatment period was divided in two consecutive sequences: during the first 6 weeks, treatment was applied once a day on an inpatient basis, whereas during the last 12 weeks, it was applied twice a week, and patients were allowed to return home if the ulcer progression was satisfactory. Patients were encouraged to keep the foot with the ulcer totally nonweight-bearing until the study was completed, but no specially designed footwear was prescribed.

Table 1—Baseline characteristics of the patients

	Group P (Placebo-Treated) n = 8	Group F (bFGF-Treated) n = 9
Age (years)	63.6 ± 7.9	61.9 ± 10.0
Sex (M/F)	7/1	9/0
Body mass index (kg/m <sup>2</sup> )	29.3 ± 2.6	26.4 ± 4.6
HbA <sub>1c</sub> (%)	7.1 ± 1.7	7.9 ± 1.7
Fructosamine (mmol/l)	284.4 ± 42.2	295.1 ± 75.0
Duration of diabetes (years)	18.8 ± 9.5	20.9 ± 12.3
Vibration perception threshold (V)	37.3 ± 14.9	46.3 ± 6.4
Ulcer duration (months)	27.9 ± 42.2	22.4 ± 27.9
Ulcer largest diameter (mm)	18.1 ± 6.2	18.0 ± 12.0
Wagner's classification		
Grade 1	1	2
Grade 2	4	4
Grade 3	3	3

Data are means ± SD. No statistically significant difference was found between groups P and F. Vibration perception threshold was measured at the great toe.

After randomization, eight patients were treated with placebo (group P) and nine received bFGF (group F). There were no significant differences in patient characteristics at entry into the study (Table 1). Glycemic control was similarly good in the two groups, due to the initiation of the intensive insulin therapy before inclusion in the protocol. The distribution in Wagner's grades and the initial size of ulcers did not differ between groups P and F.

bFGF was produced by recombinant DNA technology using *Escherichia coli* type  $\beta$ . A stable pharmaceutical formula was obtained, having a potent biological activity both in vitro and in vivo. From a lyophilate containing 50  $\mu$ g of bFGF, formula was reconstituted with saline at a final concentration of 5  $\mu$ g/ml in a vial connected in a sterile manner to a special spray delivery system: a volume of 50  $\mu$ l containing 500 ng of compound was delivered at each pressure, so that at 2 cm from the skin, the device delivered spray on a 4.15 cm<sup>2</sup> area. When reconstituted, the solution was kept at 4°C for no more than 3 days. After mechanical removal of necrotic tissue and excision of surrounding callosities, placebo or bFGF

was sprayed on the ulcer. According to the ulcer size, one or two sprays were delivered. Afterward, the ulcer was covered with a sterile petrolatum-impregnated gauze (Tulle gras Lumière, Sarbacq, Suresnes, France) in Le Grau du Roi center or dry compresses at Montpellier, until the next foot care. No antiseptics or special dressings were used.

Patients were evaluated each week: the length and width of the ulcer were measured and a photograph was taken, using an instant camera with a magnification of 1:1 (ImagePro, Polaroid, Cambridge, MA). The ulcer and periwound area were evaluated for infection and treatment tolerance. At the end of the study, each photograph was coded and digitized (Mac IIx computer, Epson GT 4000 scan, and Epscan Mac 102 software); using Canvas software, ulcer perimeter and area were measured by one of us (J.P.D.), unaware of the patients' identity, date of photographs, and nature of the treatment. Measurements were standardized in reference to the distance between the base of the big and the fifth toe. The rate of wound healing was calculated from the change in wound perimeter and area; the rate of linear advance of healing

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toward the ulcer center ( $\delta$ , in mm) was calculated according to Gilman's formula (18).

Clinically, improvement of ulcer aspect was defined by a decrease of Wagner's grade, worsening by an increase and healing by grade 0.

At entry and at the end of the study, blood was withdrawn for determination of glycosylated hemoglobin, fructosamine, and hematological and biochemical routine parameters.

Statistical analysis was performed using BMDP statistical software (19). Fisher's exact test and the nonparametric Kruskal-Wallis test were used to compare qualitative and quantitative data, respectively, between groups. In each group, comparisons of data at entry and at the end of the study were carried out using Wilcoxon's exact rank-sum test and McNemar's test for dependent quantitative and qualitative data, respectively. Multivariate analysis of variance for repeated measures was performed to determine the effect of treatment and placebo on changes in standardized wound area over time. Times until 25 and 50% healing were analyzed according to Kaplan-Meier estimates of the healing time distribution; Breslow-Mantel's test was used to adjust for the subset of the prognosis variables. The stepwise Cox proportional hazards model was used to identify and control for these prognosis variables, when statistically significant. Linear correlation and analysis of covariance were performed to control for the effect of the initial wound diameter on Gilman's parameter.

**RESULTS**—During the study, good glycemic control was maintained in both groups; at the end of the study, the level of glycosylated hemoglobin was  $6.4 \pm 1.1\%$  in group P and  $7.5 \pm 1.8\%$  in group F (NS) and that of fructosamine was  $281 \pm 48$  mmol/l in group P vs.  $311 \pm 40$  mmol/l in group F (NS). These levels were not significantly different from those at entry.

In placebo-treated patients, five of eight ulcers (63%) healed completely

Table 2—Ulcer evolution during treatment

	Group P (n = 8)	Group F (n = 9)
Treatment duration	64.8 $\pm$ 29.5	87.7 $\pm$ 38.0
Clinical outcome		
Healing	5	3
Improvement	1	2
No progression	0	2
Worsening	2	2
Ulcer perimeter reduction (% of initial perimeter)	47.2 $\pm$ 36.4	35.8 $\pm$ 49.6
Time for 50% of healing (weeks)	5.8 $\pm$ 0.4	9.3 $\pm$ 2.1

Data are means  $\pm$  SD. No statistically significant difference was found between groups P and F.

compared with three of nine (33%) in the bFGF-treated group (NS) (Table 2). In seven of eight healed ulcers, healing occurred before the end of the study. Infection occurred on two ulcers in both groups, requiring the study to be stopped prematurely. One bFGF-treated patient refused to continue the study despite improvement in her ulcer status. In five patients, the study was conducted to the end: two ulcers improved but only one in group F healed completely. No significant difference was found between the two groups with regard to the duration of treatment. In addition, the number of patients having pursued the study beyond the 6th week was identical in groups P and F. The clinical evolution of the ulcers was not significantly different; repartition in Wagner's classification was similar in both groups at the end of the study.

According to standardized measures from photographs, no significant difference was found between the two

groups for ulcer sizes at any week. In both groups, ulcer perimeter and area decreased significantly during the study. Decrease of ulcer perimeter and area was greater in group P, as was the daily reduction, but the difference was not statistically significant (Table 3). The same trend was also found when reduction of ulcer size was expressed as percentage of initial size (Tables 2 and 3).

Kaplan-Meier curves of times needed for ulcers to achieve 50 and 100% healing were plotted. Cox regression indicated no statistically significant effect because of treatment groups, centers, body mass index, patients' age, or ulcer duration. Mean healing times were identical for both groups (Table 2), and the median for 50% healing was  $9.3 \pm 2.1$  and  $5.8 \pm 0.4$  weeks for groups F and P, respectively (NS). Median time to 100% healing could not be compared because of the few events (<50% of ulcers com-

Table 3—Ulcer area reduction in the study groups

	At entry (cm <sup>2</sup> )	At the end (cm <sup>2</sup> )	Reduction (cm <sup>2</sup> )	Reduction (% initial area)	Daily reduction (mm <sup>2</sup> /day)
Group P					
Mean	0.54	0.27	0.31	75.0	0.56
SD	0.37	0.54	0.24	39.1	0.52
Group F					
Mean	0.69	0.49	0.23	59.3	0.38
SD	1.27	1.14	0.20	44.5	0.54

No statistically significant difference was found between groups P and F. In cases of enlargement in wound size reduction was considered to equal 0.



pletely healed) at the end of the study in group F.

The rate of linear advance of healing was positive in the two groups, averaging  $0.116 \pm 0.129$  and  $0.053 \pm 0.048$  cm at 6 weeks (NS) and  $0.184 \pm 0.190$  and  $0.072 \pm 0.089$  cm at 11 weeks, for groups P and F, respectively ( $P = 0.08$  by Kruskal-Wallis test). No significant relationship was found between initial ulcer size and rate of linear advance of the ulcer margins.

Topical bFGF treatment was well tolerated; we observed no clinical drug-related adverse events or abnormalities in hematological or biochemical data.

**CONCLUSIONS** — The main conclusion of this study is that local application of topical bFGF is no more effective than placebo to promote healing of neurotrophic diabetic ulcer of the foot, whether healing was expressed functionally (by Wagner's grade) or quantitatively (by measurement from photographs); moreover, the only trends we found showed a beneficial effect of placebo. The small size of the groups might have obscured a positive effect of bFGF, but an acceleration of wound healing in diabetic patients was recently reported using topical application of other growth factors in a sample of comparable size to this study (20). Of the eight healed ulcers (five in group P, three in group F), seven occurred during the hospital stay, when all patients were at bed rest, emphasizing the importance of keeping weight off of ulcer sites in promoting healing of neuropathic ulcers (2-4); this simple measure probably accounts for the good results obtained with placebo alone. A difference in compliance to non-weight-bearing recommendations between the two groups after hospital discharge seems unlikely to account for the failure of bFGF to accelerate healing. Moreover, the number of patients studied beyond the 6th week as outpatients was identical in the two groups, and the only ulcer that healed during the outpatient period was observed in group F. Diabetes control also is

essential to promoting ulcer healing (2). In this study, blood glucose control was similarly good in both groups as evidenced by glycohemoglobin and fructosamine levels; thus, the deleterious role of external factors, mechanic or metabolic, can be ruled out in accounting for the poor results with bFGF.

The result of this trial is rather disappointing, considering the physiological role of bFGF in the healing process and the previous *in vivo* and *in vitro* experiments using bFGF.

Diabetes mellitus is characterized by failure of wounds to heal. It has been hypothesized that this defect may result from the decrease or unavailability of growth factors at the ulcer site (21). Thus, topical application of growth factors seems to be a logical means to promote wound repair in diabetic patients. Among growth factors, one of the most widely studied is bFGF, a heparin-binding single-chain peptide of 146 amino acids, with a ubiquitous distribution in mesoderm- and neuroectoderm-derived tissues (13). bFGF is a potent mitogen for all cell types involved in the wound-healing process and is highly angiogenic and also chemotactic for fibroblasts and endothelial cells (22). Thus, bFGF appears to play a major role in wound healing, acting at almost all the steps of this complex process, and may represent a healing-promoting agent of potential value.

*In vivo* studies in uncompromised animal models showed that topical bFGF did accelerate healing (12,23). More relevant to our study are the results obtained in diabetic animals. In *db/db* mice, it was reported that topical application of bFGF resulted in an improvement in tissue repair (24,25).

Clinical trials on the effect of growth factor application on the healing of chronic diabetic wounds remain scarce and controversial; the most extensive clinical experience dealt with the so-called platelet-derived wound-healing formula (PDWHF), a homologous blood platelet releasate containing several growth factors, mainly platelet factor 4,

platelet-derived growth factor (PDGF), transforming growth factor  $\beta$ , and  $\beta$ -thromboglobulin-related peptides (26). In randomized, double-blind, placebo-controlled trials, topically applied PDWHF significantly accelerated the wound-healing rate of chronic diabetic, mainly neurotrophic, foot ulcers (20,26). In contrast, topical PDGF applied in 25 diabetic patients with neuropathic ulcers did not result in a significant acceleration of the healing rate (27). In addition, it was recently reported that bFGF enhanced slightly, but nonsignificantly, healing of venous and diabetic ulcers in a double-blind, randomized trial (31). Thus clinical trials using growth factors have shown discrepant results. The poor effect of bFGF in the present trial may be explained in the light of the conflicting data of these previous studies: the topical application of a single growth hormone would be insufficient to promote healing in diabetic neuropathic ulcers, as suggested by some experimental data (29). Moreover, it was reported that topically applied epidermal growth factor and insulin acted synergistically to improve wound healing in diabetic rats (30). Thus, if a single growth factor may accelerate tissue repair in the absence of profound healing defect (32,33), a "cocktail" of several growth factors might be required to induce the same beneficial effect in chronic healing deficiency states such as diabetes mellitus.

The dose of bFGF locally delivered on ulcer sites may also be questionable. In this study, doses ranging from 0.25 to 0.75  $\mu\text{g}/\text{cm}^2$  were delivered at each application on the ulcer site, lower than those used in the successful treatment of pressure sores (29). Nevertheless, the dosage used in our trial was based on previous animal studies demonstrating the efficacy of such doses to promote healing in various clinical conditions, including corneal ulcers (33). On the other hand, bFGF was applied only twice a week during the last 12 weeks, which may explain the poor result beyond this time point. From this point of view, it is

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worth noting that the efficacy of PDWHP in healing diabetic foot ulcers was obtained by daily application of the formula for 20 weeks (20,26). The last point deserving consideration is the galenic formulation of bFGF, given the fact that the product we applied was a simple collyrium. As bFGF, it might be locally degraded and/or adsorbed into the dressing, losing part of its eventual efficacy. Indeed, it was recently reported that the concentration of bFGF, in the same formulation we used, decreased by ~50% 4 h after contact with sterile gauze (34). Thus, it is possible that incorporating bFGF into a gel or a cream might result in a significant effect.

In summary, the results from this pilot study do not demonstrate significantly improved healing of neuropathic ulceration of the foot in diabetic patients treated by topically applied bFGF compared with placebo. The lack of a beneficial effect of bFGF might be explained by the profound healing defect in diabetes mellitus, requiring the combination of several locally applied and specially formulated growth factors on a daily basis.

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*Levin and O'Neal's*  
**THE  
DIABETIC  
FOOT**

**S I X T H E D I T I O N**

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## Chapter

## 18

MODULATING WOUND  
HEALING IN DIABETES

■ David L. Steed

Diabetic foot ulcers are a serious problem that may lead to infection and loss of limb. There are between 11 and 16 million patients with diabetes in the United States. Ten percent of diabetic patients may be at risk for the development of foot ulcers. Although 6 to 10% of hospitalizations in diabetic patients are for management of foot ulcers, these admissions account for nearly 25% of the hospital days in this patient group. There are 50,000 to 60,000 amputations performed in diabetic patients each year in the United States. The cost to society of caring for these patients is billions of dollars.

Diabetic foot ulcers occur because of neuropathy and peripheral vascular disease. Sixty to 70% of diabetic patients with foot ulcers have peripheral neuropathy as the etiology, while 15 to 20% have peripheral vascular disease as the cause, and another 15 to 20% have both. The neuropathy occurs as a complication of prolonged glucose elevation. It may be present in as many as 10% of patients when diabetes is diagnosed and nearly half of diabetic patients who have had their disease for more than 20 years. Peripheral neuropathy has both sensory and motor components. The sensory neuropathy leads to a loss of protective sensation in the foot. The motor neuropathy affects the nerves that control motion of the foot. Paralysis of the

intrinsic muscles of the foot leads to a "claw" deformity of the foot where the toes are pulled up and do not touch the ground. This causes the metatarsal heads to become more prominent on the plantar surface and reduces weight bearing from the toes. The metatarsal heads are a common site of plantar ulceration in diabetic patients. This is especially true for the areas beneath the first and fifth metatarsal heads. The patients can also develop midfoot collapse with loss of the plantar arch due to fractures and fracture dislocations. This Charcot neuroarthropathy may result in a markedly abnormal shape to the foot, which coupled with the sensory neuropathy, makes the diabetic patient at risk for skin breakdown.

There are other factors that lead to ulceration in diabetic patients. The patients commonly have peripheral vascular disease. Diabetes is a commonly accepted risk factor for atherosclerosis as are smoking, hyperlipidemia, and hypertension. Although there was once debate as to whether diabetic patients had "small vessel disease" with occlusion of very small vessels, that theory has not been upheld. Most would agree that diabetic patients are at increased risk for typical atherosclerosis. They do seem to develop a pattern of disease commonly involving the tibial arteries. Other problems in diabetic patients

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also complicate the problem of foot ulceration such as some thinning of the skin of the plantar surface of the foot and an inability to deal well with infection.

### Wound Healing

Wound healing is the process by which tissues respond to an injury. The biologic process is complex and involves chemotaxis, cell replication, production of proteins, neovascularization, maturation, and wound remodeling. Wound repair and regeneration occur in an orderly and predictable manner and are under the control of growth factors, which though present in the body in only small amounts, exert a strong influence on wound repair.<sup>13</sup> Growth factors are polypeptides that regulate the growth, differentiation, and metabolism of cells and direct the process of tissue repair.

Wound healing occurs in three phases: inflammation, fibroplasia, and maturation. Each of these phases is controlled by growth factors. The inflammatory response begins immediately after injury. Vasoconstriction limits hemorrhage within the site of wounding.<sup>20</sup> Damage to the endothelial surface of arteries and veins allows blood to leak from the vessel wall, exposing platelets to collagen within the media of the vessel wall. The coagulation cascade is initiated by these platelets. Serotonin and thromboxane are released to enhance vasoconstriction locally to keep healing factors within the wound space. At nearly the same time, vasodilatation occurs at adjacent sites to allow new factors to be brought into the wound. This vasodilatation is mediated by histamine, and released by platelets, mast cells, and basophils. Vascular permeability increases to allow these blood-borne factors to enter the site of wounding.

Platelets activate the coagulation cascade through both the intrinsic and extrinsic response. The intrinsic response is mediated through Hageman factor, factor XII, as it comes into contact with collagen. In the presence of kininogen, a precursor of bradykinin and prokallikrein, factor XII activates factor XI then factor IX, then factor VIII. The extrinsic system is activated through thromboplastin formed when phospholipids and glycoproteins are released by blood coming into contact with the injured tissues. In the presence of calcium, factor VII is activated. Both

the intrinsic and extrinsic systems stimulate the final common pathway leading to fibrin production and polymerization. Simultaneously, the fibrinolytic system is activated to monitor clotting so as to prevent coagulation from extending beyond the wound space.<sup>27</sup> It is controlled by the same factors that initiate coagulation and thus serves as a regulator of the process. Arachidonic acid is produced and serves as an intermediate for the production of prostaglandins and leukotrienes.

These intense vasodilators act with histamine, bradykinin, and complement to increase vascular permeability. Thromboxane also increases platelet aggregation and local vasoconstriction. The complement cascade is activated at the same time by platelets and neutral proteases to produce very potent anaphylotoxins, which cause mast cells to degranulate and release histamine. This process leads to margination and then migration of white blood cells into the wound space. The neutrophils are phagocytes for bacteria. Although wounds can heal without white blood cells, the risk of infection is increased. Other substances released by the inflammatory process are also chemoattractants for neutrophils, which produce free oxygen radicals and lysosomal enzymes for host defense. The neutrophils are later removed from the wound by tissue macrophages.

Monocytes migrate into the wound space by the third day and become tissue macrophages. Wounds cannot heal without the macrophage. These cells control and regulate the wound environment through the production and regulation of growth factors. These growth factors control the cellular composition of the wound as well as matrix formation and remodeling. Extracellular matrix is a variety of proteins in a polysaccharide gel composed of glycosaminoglycans and proteoglycans produced by fibroblasts. Matrix proteins may be structural such as collagen and elastin, while others such as fibronectin and laminin regulate cell adhesion. Thrombospondin, von Willebrand factor, and laminin are also adhesion molecules. Fibronectin is also a chemoattractant for circulating monocytes and stimulates their differentiation into tissue macrophages.<sup>11</sup>

Macrophages and fibroblasts enter the wound to begin the process of fibroplasia, the second phase of wound healing. This process begins around the fifth day following injury and may continue for 2 weeks. The inflammatory response lessens as the mediators of

inflammation are no longer produced, and those present are inactivated or removed by diffusion or by macrophages. Fibroplasia is the process of matrix formation including collagen synthesis. Angiogenesis is critical to this phase of wound healing to bring a blood supply into the wound. Fibroblasts are attracted to the wound and replicate in response to fibronectin; platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF), and C5a, a product of the complement system. These fibroblasts produce proteoglycans and structural proteins necessary for wound healing. This matrix is composed of hyaluronate and fibronectin, which allow for cellular migration stimulated by chemotactic factors formed in the wound. Fibronectin is also important in epithelialization and angiogenesis.

Collagen, the major structural protein in the wound, is the most common protein in the mammalian world. Produced by the fibroblast, it is a family of at least 12 proteins, rich in glycine and proline and bound in a tight triple helix. Cross-linking between the three strands results in a very stable molecule resistant to breakdown. The production and release of collagen from fibroblasts is controlled by growth factors such as PDGF, epidermal growth factor (EGF), FGF, and TGF- $\beta$  produced by the tissue macrophage. Once deposited in the wound, collagen is then remodeled for several years. Elastin is another structural protein in the wound present in much smaller amounts than collagen. It contains proline and lysine. It is configured as random coils, allowing both stretch and recoil. Angiogenesis is the process of capillary formation from the budding of existing capillaries after stimulation by FGF.<sup>9, 24</sup> These capillaries are composed of endothelial cells that migrate through the healing wound. Connections are developed between the capillaries to form a network in the wound space. This capillary network provides a pathway for new healing factors to enter the wound. The process ceases when the wound has an adequate blood supply. It appears as if the process of angiogenesis is controlled by oxygen tension and is stimulated by hypoxia. Epithelialization occurs only after granulation tissue is established. Cells migrate from the edge of the wound over the collagen-fibronectin surface. This process results in mature skin covering the wound.

Scar contracture occurs as the wound matures. During the maturation process, the scar becomes less hyperemic as blood supply is reduced. Although remodeling and wound strengthening increase for up to 2 years following injury, the total collagen content of the wound does not change. Hyaluronidase, collagenase, and elastase are key elements in wound remodeling. Hyaluronate is replaced by dermatan sulfate and chondroitin sulfate, which reduce cell migration and allow those cells already in the wound space to differentiate. Plasmin formed from plasminogen degrades fibrin. Collagenase is secreted by macrophages, fibroblasts, epithelial cells, and white blood cells and is able to break the collagen triple helix to allow remodeling. Urokinase, produced by leukocytes, fibroblasts, endothelial cells, and keratinocytes, activates collagenase and elastase.

### Growth Factors

The process of wound healing is controlled by growth factors, polypeptides that initiate cell growth and proliferation and protein production by binding to specific high-affinity receptors on the cell surface. They have the ability to stimulate mitosis of quiescent cells. They commonly have endocrine, paracrine, and autocrine function. Some are transported in plasma bound to large carrier proteins, while others affect nonadjacent cells or may have a self-regulating effect. They are produced by platelets, macrophages, epithelial cells, fibroblasts, and endothelial cells. Although growth factors are present in only minute amounts, they modulate the process of wound repair. The growth factors most commonly involved in wound healing include PDGF, TGF, EGF, FGF, and insulin-like growth factor (IGF).

### Platelet-Derived Growth Factor

Platelets that initiate the coagulation cascade in the wound are the initial source of growth factors including PDGF, TGF- $\beta$ , EGF, and IGF-I. Other cells drawn into the wound space such as inflammatory cells, fibroblasts, and epithelial cells are also involved in growth factor production. Growth factors are chemoattractants for neutrophils, macrophages, fibroblasts, and endothelial cells. Macrophages release factors such as



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tumor necrosis factor (TNF). Wound remodeling occurs under the control of collagenase, which is produced in response to EGF, TNF, interleukin-1 (IL-1), and PDGF. Thus, the complete process of wound repair is controlled directly or indirectly by growth factors.

PDGF is the most widely studied growth factor clinically. It is produced by platelets, macrophages, smooth muscle cells, vascular endothelium, and fibroblasts.<sup>18</sup> PDGF has a molecular weight of 24,000 daltons and is composed of two chains, A and B, held together by disulfide bonds in three dimeric forms, AA, AB, and BB. There is a 60% amino acid homology between the two chains. Human platelets contain all three forms of PDGF in a ratio of about 12% AA, 65% AB, and 23% BB.<sup>1</sup> The B chain is quite similar to the transforming gene of the simian sarcoma virus. The human proto-oncogene C-sis is similar to the viral oncogene V-cis and encodes for the B chain of PDGF. There are two PDGF receptors, an  $\alpha$ - and a  $\beta$ -receptor.<sup>23</sup> The  $\alpha$ -receptor recognizes both the A and B chains of PDGF and thus can bind to the AA form, the AB form, and the BB form. The  $\beta$ -receptor recognizes only the B chain, and thus binds only to the BB form and weakly to the AB form. Most cells have many times more  $\beta$ -receptors than  $\alpha$ -receptors. Cells with PDGF receptors include fibroblasts, vascular smooth muscle cells, and some microvascular endothelial cells.

PDGF is a mitogen for cells of mesenchymal origin. PDGF is a potent chemoattractant and mitogen for fibroblasts, smooth muscle cells, and inflammatory cells. It also acts with TGF- $\beta$  and EGF to stimulate mesenchymal cells. Although PDGF is produced by endothelial cells, they do not respond to PDGF but work in a paracrine manner to stimulate adjacent vascular smooth muscle. Smooth muscle cells also act in an autocrine fashion and produce PDGF.

PDGF is stable to extremes of heat, a wide range of pH, and degradation by proteases. Platelets are the largest source of PDGF in the human body. There are no cases of a human PDGF deficiency state, perhaps suggesting that PDGF is critical to the survival of the individual.

PDGF has been isolated from the  $\alpha$ -granules of platelets and has been produced through recombinant DNA technology. In animal models, it has been shown to improve the breaking strength of incisional wounds in

rats when applied topically even as a single dose.<sup>23</sup> PDGF accelerates wound healing; however, by 3 months, there is no difference in wound healing as compared with untreated wounds, suggesting that wound healing stimulated by PDGF is quite similar to normal healing. Wounds treated with PDGF had a marked increase in neutrophils, monocytes, and fibroblasts in an animal model.<sup>21</sup> This cellular response leads to an increase in granulation tissue production. Similar findings were observed in a rabbit ear excisional wound model. Despite the fact that PDGF does not have a direct effect on keratinocytes, wounds in animals were shown to have an increase in epithelialization. This is due to the influence of macrophages and fibroblasts attracted into the wound by PDGF. Wounds in animals treated topically with PDGF have an increase in neovascularization, although PDGF does not directly stimulate endothelial cells. Again, this is likely related to the influence of other cells attracted into the wound by PDGF. Thus it appears that PDGF accelerates wound healing by accelerating the normal responses. The healed wounds appear to be normal in all aspects.

PDGF has been studied in several clinical trials. The effectiveness of recombinant human PDGF-BB in healing decubitus ulcers was evaluated in patients treated and followed for 28 days.<sup>24</sup> There appeared to be greater reduction in wound closure in patients treated with PDGF. In another trial, patients with decubitus ulcers were treated with PDGF or placebo for 1 month.<sup>20</sup> The ulcer volume was significantly reduced in the PDGF-treated patients. No significant toxicity related to PDGF was noted in either study. A randomized, prospective, double-blind trial of recombinant human PDGF-BB was performed in patients with diabetic neurotrophic foot ulcers.<sup>20</sup> Patients were treated with PDGF (at a dose of 2.2  $\mu\text{g}/\text{cm}^2$  of ulcer area) in vehicle, carboxymethylcellulose, or vehicle alone for up to 20 weeks or until complete wound closure had been achieved. All wounds had been present for at least 8 weeks. All patients were free of infection and had an adequate blood supply as demonstrated by a transcutaneous oxygen tension ( $\text{TcPO}_2$ ) of a least 30 mm Hg. Wounds were debrided prior to entry into the study and as needed during the trial. Forty-eight percent healed using PDGF, while only 25% healed using vehicle alone ( $p < 0.01$ ). The median reduc-

tion in wound area was 98.8% for PDGF-treated patients but only 82.1% for those treated with vehicle. There were no significant differences in incidence or severity of adverse events in either group. This was the first study to suggest that a growth factor, and specifically PDGF, could be applied topically and be effective and safe in promoting the healing of chronic wounds in humans. In another trial using recombinant human PDGF in the treatment of patients with diabetic foot ulcers, PDGF-BB was found to increase the incidence of complete wound closure by 43% as compared with placebo ( $p = 0.007$ ).<sup>34</sup> It also decreased the time to achieve complete wound closure by 32% ( $p = 0.013$ ) when compared with the placebo gel. Over 1,000 patients treated with PDGF have been reported. It appears to be safe and efficacious and is now approved for use in the treatment of diabetic foot ulcers. It has been noted that those patients receiving the best wound care healed better when treated with PDGF. Debridement was found to be critically important.<sup>35</sup> The benefits from PDGF will not be achieved if the wounds are not treated properly.

### Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is quite similar to PDGF. It has a molecular weight of 45,000 daltons. Although it has a 24% amino acid homology to the B chain of PDGF, it binds different receptors than PDGF and has different actions.<sup>17</sup> VEGF is a potent mitogen for endothelial cells but not for fibroblasts or vascular smooth muscle cells as is PDGF. Thus, VEGF is angiogenic and plays a role in wound healing through this mechanism. There has been interest in using VEGF to stimulate the development of collateral vessels in patients with symptomatic arterial ischemic disease. VEGF may be delivered to the ischemic area as a protein or through gene transfer.

### Fibroblast Growth Factor

Fibroblast growth factor is a series of heparin-bound growth factors.<sup>10</sup> There are two forms of this growth factor, acidic FGF (aFGF) and basic FGF (bFGF). Both are single-strand molecules with molecular weights of 15,000. There is a 60% amino acid

homology between the two molecules. The binding of these molecules to heparin or heparan sulfate may protect them from enzymatic degradation. Both aFGF and bFGF are found in the extracellular matrix in the bound form. Matrix degradation proteins may act to release aFGF or bFGF. Acidic FGF is similar to endothelial cell growth factor, while bFGF is similar to endothelial cell growth factor II. Both aFGF and bFGF are similar to keratinocyte growth factor. FGFs are produced by fibroblasts, endothelial cells, smooth muscle cells, and chondrocytes and are mitogens for cells of mesodermal and neuroectodermal origin. FGFs are also potent mitogens for endothelial cells and act as angiogenesis factors by stimulating growth of new blood vessels through proliferation of capillary endothelial cells.<sup>6</sup> In addition to endothelial cells, FGFs can stimulate fibroblasts, keratinocytes, chondrocytes, and myoblasts. Multiple different FGF receptors have been identified. They appear to have a similar function. To date, there are no clinical trials that have proven FGF to be of benefit in clinical wound healing.

### Keratinocyte Growth Factor

Keratinocyte growth factor (KGF) is related to the FGFs. It is a protein with a molecular weight of 28,000 and has a significant amino acid homology with the FGFs.<sup>6</sup> Although KGF is found only in fibroblasts, it stimulates keratinocytes, not fibroblasts. It may share a receptor with FGF. At this time, there are no clinical trials reported using KGF, and its role in human wound healing remains to be defined.

### Transforming Growth Factors

TGFs are composed of two polypeptide chains,  $\alpha$ - and  $\beta$ .<sup>36</sup> TGF- $\alpha$  has a 30% amino acid homology with EGF. It received its name because of its ability to reversibly stimulate the growth of cells. Cancer cells do this as well. TGF- $\alpha$  is produced by macrophages, keratinocytes, hepatocytes, eosinophiles, and other cells. TGF- $\alpha$  and EGF are mitogens for keratinocytes and fibroblasts but TGF- $\alpha$  is a more potent angiogenesis factor. Both TGF- $\alpha$  and EGF bind to the EGF receptor but their specific actions may be different partly due to differences in their binding to

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the receptor. To date there have been no clinical trials of wound healing with TGF- $\alpha$ .

TGF- $\beta$  has no amino acid homology with TGF- $\alpha$  or any other growth factor. TGF- $\beta$  is a molecule with many different functions and can stimulate or inhibit the growth or differentiation of many different cells. It is, thus, in some respects a master growth factor. There have been three different forms of TGF- $\beta$  isolated, TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3.<sup>10</sup> Just as there are three forms of TGF- $\beta$ , there are three receptors, although the three are not equally important. The actions of the three different forms of TGF- $\beta$  are very similar although not exactly the same. TGF- $\beta$ s have a molecular weight of approximately 25,000. TGF- $\beta$  is a group of proteins that can reversibly inhibit growth of cells, especially those of ectodermal origin. TGF- $\beta$  is produced by a variety of cells including platelets, macrophages, fibroblasts, keratinocytes, and lymphocytes. Nearly all cells have receptors for TGF- $\beta$  and have the potential to respond to it. It appears as if TGF- $\beta$  is the most widely acting of the growth factors. The TGF- $\beta$ s are potent stimulators of chemotaxis in inflammatory cells and stimulate cells to produce extracellular matrix. These characteristics make the family of TGF- $\beta$ s important to the wound healing process. There have been trials of TGF- $\beta$  in the treatment of psoriasis, but as yet, there are no trials reported using TGF- $\beta$  in human wound healing.

### Epidermal Growth Factor

EGF is a small molecule quite similar to TGF- $\alpha$ , with a molecular weight of 6,200 daltons. EGF is produced by the platelet and is present in large amounts in the early stages of wound healing. EGF is produced by the kidney, salivary glands, and lacrimal glands; therefore, it is found in high concentrations in urine, saliva, and tears.<sup>1</sup> EGF promotes epidermal regeneration and corneal epithelialization, and increased the tensile strength of wounds in animals. EGF increases wound healing by stimulating the production of proteins such as fibronectin and the migration of epithelial cells. Although EGF does not stimulate collagen production, it increases the number of fibroblasts in the wound. These cells produce collagen and improve the wound strength. EGF has a common receptor

with TGF- $\alpha$ . EGF has been studied in a randomized trial of skin graft donor site healing. Treatment of donor sites with silver sulfadiazine containing EGF accelerated epidermal regeneration compared with sites treated with silver sulfadiazine alone.<sup>5</sup> EGF reduced the healing time by 1.5 days. Although these results may not be clinically significant, this was the first trial to suggest a benefit from treatment with an isolated growth factor in human wounds. EGF was also used in a prospective open label trial in patients with chronic wounds.<sup>1</sup> Chronic wounds were treated with silver sulfadiazine. In this crossover dosing, those who did not heal were then treated with silver sulfadiazine containing EGF. Many of the patients improved. The results from these studies suggest that EGF may be beneficial in wound healing, although there is not adequate proof as yet to confirm this premise.

### Insulin-Like Growth Factors

IGFs, also called somatomedins, are proteins sharing a 50% amino acid homology with pro-insulin. They have insulin-like activity. The two forms of this growth factor, IGF-1 and IGF-2, are both secreted as large precursor molecules, which are cleaved to an active form.<sup>25</sup> IGF-1 is also called somatomedin-C while IGF-2 is simply somatomedin. Many tissues synthesize these growth factors. IGF-1 can be found in the liver, heart, lung, pancreas, brain, and muscle.<sup>2</sup> Although produced by a number of tissues, IGF-2 is particularly prominent during fetal development and plays a significant role in fetal growth. IGF-1 and IGF-2 have separate receptors. The actions of pituitary growth hormone are mediated through IGF-1. Pituitary growth hormone stimulates cell differentiation and the production of IGF-1. IGF-1 then causes cell division. IGF-1 is produced mainly in the liver. It is found in high concentrations in platelets and is released into the wound space when clotting occurs. Levels of IGF-1 and IGF-2 depend on multiple factors, such as age, gender, nutritional status, and hormone level. Growth hormone regulates IGF-1 and IGF-2 levels as does prolactin, thyroid hormone, and the sex hormones. Elevated levels of somatomedins have been identified in patients with acromegaly.

IGF-1 and IGF-2 are anabolic hormones that stimulate the synthesis of glycogen, pro-

tein, and glycosaminoglycans. They increase the transport of glucose and amino acids across cell membranes. They also increase collagen synthesis by fibroblasts. There is no clinical evidence to suggest these growth factors are of benefit in treating wounds.

As previously described, the process of wound healing is complex and involves platelets and macrophages. In the first 2 days following injury, platelets control the wound space by way of growth factors that they produce and release. Following this period, this function is taken over by macrophages. Within the  $\alpha$ -granules of the human platelet are multiple growth factors that are released when platelets are activated and degranulate. These include PDGF, TGF- $\beta$ , FGF, EGF, platelet factor 4, platelet-derived angiogenesis factor, and  $\beta$ -thromboglobulin.

### Platelet Releasates

A purified platelet releasate has been prepared by stimulating human platelets to release the contents of their  $\alpha$ -granules by using thrombin. Use of a platelet releasate in wound healing has several advantages. First, the growth factors that are released are identical to and in the same proportion as the growth factors normally brought in the wound space by the platelet. Second, preparation of a platelet releasate is relatively easy and inexpensive, as the platelets can be harvested from peripheral blood. They readily release the contents of their  $\alpha$ -granules when stimulated with thrombin. As growth factors are preserved in banked blood, large amounts can be retrieved from the platelets of pooled human blood. There are, however, disadvantages to using a platelet releasate. Not all growth factors promote healing of a wound. It seems reasonable to assume that there is a signal for wound healing to stop. It is likely that this, too, is growth factor related. Since the proper concentration of a platelet releasate is uncertain, a platelet releasate might concentrate factors promoting healing as well as those that trigger the wound healing process to end. Another major disadvantage of such a preparation is that there is the possibility of transmission of an infectious agent from the platelet donor. This could be minimized if the releasate were harvested from a single donor.

### Clinical Trials

There have been several reports using a platelet releasate in wound healing. A preliminary report described the use of an autologous platelet releasate in six patients with chronic lower extremity ulcers from connective tissue diseases with minimal benefit. A homologous platelet releasate was used to treat 11 patients with leg ulcers from diabetes and eight patients with leg ulcers secondary to chronic venous insufficiency.<sup>11</sup> No benefit was observed from the treatment with a platelet releasate. In managing these patients, treatment of the underlying disease was not addressed. Growth factors cannot be expected to improve wound healing unless they are applied in a comprehensive wound care program that addresses the underlying etiology of these wounds such as diabetes, venous hypertension, or ischemia. In another trial, 49 patients with chronic nonhealing cutaneous wounds were treated with an autologous platelet releasate.<sup>15</sup> There was some improvement in achieving complete wound healing. This was the first clinical trial to suggest a benefit from a platelet releasate applied topically to promote the healing of chronic wounds. A randomized trial comparing platelet releasate versus a placebo was conducted in patients with ulcers secondary to diabetes, peripheral vascular disease, venous insufficiency or vasculitis.<sup>14</sup> Although this study suggested a benefit from the treatment, the growth factor preparation was added to microcrystalline collagen, a potent stimulator of platelets. The exact contribution from the collagen to the healing of these wounds was not clear. Two other trials suggested a benefit from a platelet releasate. In one trial, patients were treated for 3 months with saline and silver sulfadiazine. Only 3 of the 23 lower extremity wounds healed; however, when the platelet releasate was then applied, the remaining ulcers healed.<sup>1</sup> Thirteen patients with diabetic neurotrophic ulcers were enrolled in a randomized trial of a platelet releasate versus saline placebo.<sup>32</sup> A benefit was seen in those treated with the platelet releasate. By 20 weeks of therapy, five of seven patients healed using the platelet releasate, whereas only two of six patients healed using the saline placebo. By 24 weeks of treatment, another three of six patients in

the control group healed, suggesting that the platelet releasate stimulated more rapid healing but did not result in a greater proportion of healed wounds. Another study of 70 patients suggested a similar benefit from a platelet releasate.<sup>12</sup>

Despite this evidence that platelet releasates are of benefit, another trial observed very different results using a homologous platelet releasate. In a randomized, prospective, double-blind, placebo-controlled trial, topical platelet releasate was applied to leg ulcers due to diabetes, peripheral vascular disease, or chronic venous insufficiency. Wounds treated with the platelet releasate worsened, while wounds in the control group improved.<sup>16</sup> This study not only suggested no benefit from a platelet releasate over standard care in the management of lower extremity ulcers, but in fact intimated that a platelet releasate might be detrimental to wound healing.

Although the results of the clinical trials using platelet releasates have been varied, there is some evidence to suggest that they may be of benefit applied topically to lower extremity wounds. However, the inconsistency of the results as well as the concern about transmission of infectious agents in using a homologous preparation leaves their role in human wound healing undefined. Molecules other than growth factors may play a role in the healing of wounds, especially in patients with diabetes. Mice deficient in inducible nitric oxide synthase (iNOS) had a slower rate of healing of full-thickness wounds than normal animals.<sup>36</sup> When given iNOS, the rate of healing returned to normal.

### Conclusion

In conclusion, growth factors control the growth, differentiation, and metabolism of cells. Thus they control wound repair and maturation, although there are only a limited number of reports where growth factors have improved wound healing. One isolated growth factor, PDGF, does improve healing in diabetic ulcers and is approved for that indication. It is likely that the actions and benefits of growth factors will be defined further. This will allow health care providers to control the wound environment to achieve complete and durable wound healing in patients.

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